

Davies, David R. 1999 B

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David R. Davies, Ph.D.

This is the second interview in a series on the career of Dr. David R. Davies. It was conducted on 10 December 1999, in his office on the third floor of Building 5, National Institutes of Health, Bethesda, Maryland. The interviewer is Dr. Buhm Soon Park.

Please note: some names unable to verify

Davies: So, Gordy was absolutely fantastic. He was witty, he was super- intelligent, he was creative. He was just a wonderful person to have around. And he was a great musician. He was equally at home playing with the NIH orchestra or playing with a group downtown. It's true. And he could play--he liked to play jazz and he liked to play classical music as well. And so, after we'd been together as a group for some six or seven years, Gordy was offered a job at Yale, and this would have been as chairman of the Biochemistry Department, which is in the medical school. And he would have replaced Fruton. Joe Fruton, who was the chairman at that time. And he then started to try and recruit some of us to go up to Yale as well. And, in fact, I remember going up once. We went up as a group, and then I went up again with him a second time. And the trouble was that Gordon was not a person who enjoyed administration. I'm choosing my words carefully now. Postdocs would arrive in the lab and people would say, "Well, who are you?" and they'd say, "Well, I'm here because Gordy offered me a job." But he'd never done the paperwork, so he'd have to hurry to patch things up. But he ran afoul of Fruton, who was a very strong-minded character, and they had some disagreements, and Gordy decided in the end that he really didn't want to go to Yale. But in the meantime, he'd set the wheels rolling, and Bruce Ames, who was there, had said, "Well, if you're thinking of leaving, then maybe it's time for me to look for a job," and he found a job in Berkeley, where he's been ever since. And Harvey Itano went to La Jolla and has been there ever since. So that was just about 1969 or so. I think it was before 1970. And then we hired two new people. Well, when Bruce left, Marty was able to get space for his lab, Marty Gellert, and when Gordy and Harvey Itano left, and various other people, who were able to get space for two new recruits, and we hired Tommy Sauer, who was a very distinguished molecular biologist from Japan, and we hired Terrell Hill, who was an even, in some ways, I would say even more, but he was also a very distinguished American molecular biologist and already a member of the National Academy and things like this, and whom we'd known from the days when he was over here at the Navy. Have I talked about the Navy at all?

Park: You talked about Navy a little bit.

Davies: A little bit, yes. Well, Terrell was one of the people over there. Then he went to California. Well, first he went to Dartmouth, I think, and then, along with the rest of them, and then they went to California. And Terrell is retired. Tommy retired. Todd Myers is retiring. But the remarkable thing is that, apart from those three guys leaving, our lab has really stayed very much the same and we've stayed together. I don't quite know why that is. I guess none of us want to build big empires. That's certainly a criterion. We had a rotating lab chief position. When Gordy left, Ed Rall was then our scientific director, and he had to decide who would be the next lab chief, and he decided that we'd be better off, since we had so many good people, in rotating the lab chief. So we did it for a year at a time.

Park: When was the first time?

Davies: Oh, this must have been back about 1970 or so. And so, up until about two years ago, we'd been doing the rotation system, one year a lab chief, four years or five years off. But now they've changed that, and Gary is now the lab chief. I think the administration felt we were too much of a moving target. They never knew who to deal with.

Park: Right, right. And that system, the rotating chief, was a very new one at NIH.

Davies: Well, I think for a long time we were the only lab that had this kind of system. And, in my opinion, it's a wonderful way to keep people together, because everything we did, we had to do by consensus.

Park: I see.

Davies: There was, you know, if there was somebody who really wanted to expand and get more space, then it would be at the expense of the other people in the lab, and it just wouldn't be possible. So it's interesting that none of us--all of us were really quite satisfied with the sources that we were given by the NIDDK, because it wasn't for lack of job offers. We were all offered lots of jobs. Another thing that I wanted to get back to right at the beginning is that I think I covered Marshall Nirenberg before.

Park: Yes.

Davies: Okay. So, of the lab that we set up in 1961, we had Harvey Itano, who had worked with Pauling on sickle cell hemoglobin; we had Bruce Ames; and we had me and Gary Felsenfeld. And did I say this? We were all at Cal Tech at the same time. I don't think I dealt with this before. Did I say that? So the lab was very much influenced by Pauling and by the sort of general atmosphere at Cal Tech at that time, plus the fact that we knew, all knew each other and had known each other since the early '50s. I mean, Gordy was, well, you know, I guess--I don't know where he got his degree probably at Harvard or somewhere. And he was the only one of the section chiefs who didn't have a strong Cal Tech association. Bruce was a graduate student there. Harvey Itano was Pauling's graduate student; Gary was Pauling's graduate student; and I was a postdoc there. So that gave us some sort of common background as well, keeping us together.

Park: And in terms of subject materials, you know, Pauling was very much interested in protein structure. Gary Felsenfeld and Alex Rich worked on

RNA structure, but gradually moved on to protein structure. And you mentioned that your experience with John Kendrew in 1959 was important for your interest in protein. Also, you mentioned that Marshall Nirenberg's discovery of the genetic code sparked, well, shifted, the view on protein studies. Could you say more about why you became more interested in proteins, and especially if there is anything related to the Cal Tech years?

Davies: Well, I mean, the big things that were coming out on proteins, certainly from a crystallographer's point of view in those days, were the alpha helix and the beta structure. And then I think I mentioned to you Alex Rich's experience in going to Cambridge, living in Francis Crick's house and working on the structure of collagen, so all these fibrous proteins were being worked out. And it was gradually realized that many of these structures appear in globular proteins as well. That took a long time. But it was happening at the same time. I mean, you know if you look at Perutz's talk, he was seeing these rods in his maps, and there was beginning to be the feeling that these probably corresponded to alpha helices. So structure was a big theme in protein science, but also in nucleic acid. I mean, there wasn't a helical structure for DNA, you know. It was such a revelation, I mean, it integrated structure and function in a way that most people would never have anticipated. So, but we were working on fiber diffraction of polynucleotides. The difference between fiber diffraction and a single-crystal diffraction of a protein is like the difference between looking at the sky on a somewhat cloudy night and looking at the sky when it's totally clear. You know, there's so much more information in a protein crystal diffraction pattern. And it was clear, I mean, with a fiber diffraction pattern, all you can really do is model oriented. You have to think of a model, and then you have to show that that model will satisfy the diffraction pattern. But you don't have a lot of information. You have maybe strong intensities up around 3.4 angstroms and you have a certain pitch to the helix that you can measure. We've assumed that it's helical. And you assume that it's a regular structure, and that's about as far as you can go. And then you look to see what the bases look like and see whether they can be arranged. Most of what we did turned out subsequently to be correct. But since I had worked at Cal Tech on very accurate structures...

Park: Yes. You showed me that.

Davies: Right. It was--this was a step in the wrong direction for me. I wanted more data and more proper crystallography, and it was becoming clear that proteins could do this, that people could crystallize proteins and they could get diffraction patterns from them. But it was still enormously labor intensive. But it was also clear that there were all these--we used to think there may be 10,000 different proteins in simple ways, and even though it's, you know, at the rate they were being done then, which was one every 10 years or so, myoglobin was the first one. So, to be able to see that electron density map and to show that there were helices inside these proteins was, I think, one of the most exciting things I ever did, and we wanted to do something like that. But when I decided to move over into proteins, there were still a huge... I was in the middle of nucleic acid work, so it took a while to get started.

Park: So, around 1965 you started working on gamma-chymotrypsin. Why did you choose to this protein?

Davies: The simple answer is that I'd seen pictures of crystals of gamma-chymotrypsin and they were beautiful and they were big, and it was crystallized at pH 6 or so, which was very close to the active pH, the peak of the active pH. And I knew that David Blow was working on alpha chymotrypsin.

Park: Who is David Blow?

Davies: David Blow is at Cambridge. And he was here recently after he gave us a little talk. But alpha chymotrypsin crystallized at pH 4, where the enzyme was quite inactive, and so we thought, well, the enzyme is inactive. I

mean, what we're doing will show a real active enzyme. That was a mistake. As it turned out, the difference was just a proton greater to a histidine or something. The structure didn't change at all. But we didn't know anything about that, so that was the first decision. The other thing was that, the other protein that I was looking at and had crystallized was subtilisin and it's another serine protease. And we had, or I had beautiful crystals, and I wanted to work on that rather than chymotrypsin. But Sid Bernhardt was section chief for the lab after Alex Rich left, and Sid was very familiar with all these proteases, and he said, "Don't work on this." He said, "When I ran it through a gel, it all came out as little bits and pieces. It's chewed itself up." So I didn't. But that was a mistake, because those crystals were wonderful. They diffracted even better than the gamma chymotrypsin crystals, and they--it turned out to be very interesting because chymotrypsin and subtilisin are examples of convergent evolution. In other words, they both have almost exactly the same active site. One is an all-beta structure with a little bit of alpha helix on the end; that's the chymotrypsin. And the other is an almost all-alpha structure. So, structurally, they're obviously derived from completely different ancestors. But it was the first real example structurally of what was clearly convergent evolution. They must have evolved quite separately and then come down to having the same function in the end. So that's why I didn't work on the subtilisin. And it was a mistake, I think, because I was competing with the huge infrastructure that had been set up at the MRC lab. They had people there who were trained to densitometer the films and record the results on paper tape. They had a big computer there, and they--it just was a mistake to work on chymotrypsin. I think I would, from a purely competitive point of view, I would have been much better working on almost anything else.

Park: You worked on chymotrypsin for four or five years?

Davies: Oh, that went on for quite a while. I think we started when Paul was still here. When Paul left, around 1964 or so, we were into it. But I was still pulling fibers of various other things. We were still working on the G structures and the polynucleotides, and so I didn't exactly charge into the protein crystallography here. I sort of eased into it more. And I think I should have pushed a little harder at that time, but I didn't. And then in the early '70s, in fact, late '60s, Bill Terry had been--I'm not sure whether Bill had taken one of my courses or what. I used to teach in the graduate school.

Park: Graduate school, FAES?

Davies: FAS, yes. I still do. And Bill Terry came to me and he said, "I'd like you to look at these cryoglobulins. Well, cryoglobulins were from patients who had multiple myeloma, and they precipitated if you cooled them. So I looked at these, and he had three or four different ones, and they were all--well, not all, but some of them were crystalline. They were very tiny crystals. So we tried to get bigger crystals. We were never able to get bigger crystals. So, what Bill did was he sent out a request to various cancer centers around the country and asked them if they had any cryoglobulins that they could let us have for the purpose of just trying to do crystallography. And we had one. I remember it very well. It was called Dobb [sp.], which I presume had to do with the name of the patient, and it came from Cincinnati, and it was described, if you put it in the refrigerator, it was described as crystallizing like rock candy, and it did. I mean, it was unbelievable. But it came with a glass vial and with some liquid in it, and if you left it in the refrigerator overnight, the next morning there would be crystals there. And, unfortunately, they didn't diffract very well. They only diffracted out to about 5 angstroms or so. That wasn't enough to solve a structure, certainly not in those days. It would have been today with all we know, but not in that time. And so I took it upstairs, where the X-ray room was, did the diffraction, and established the space group and cell dimensions. And I think Brian Matthews was in the lab then--he's now at Oregon--and Brian helped me with some of that, and so we published. We wrote a paper on it. That was the first structure. That was the first. Maybe things have changed. I think that... Yeah, that was the first report of crystalline intact antibody. Subsequently, in the next few years, Alan Edmondson worked on a structure of a protein called MCG, which was very similar to Dobb. And in Robert Hooler's [sp.] in Munich, Peter Colman [sp.] and various other people--not the K Kolemian, the C Colman from Australia... Do you know Peter?

Park: No.

Davies: C-o-l-m-a-n. He's in, he works for the CSIRO, I think, in Australia. They had a crystal of an intact protein, and the two FABs were visible in the crystal, but there was no sign of the FC, and it was clear that the hinge was so flexible that the FC could occupy a variety of different positions. And so the...

Park: Could you explain what's the FC? FABs, I know the FAB. The FC?

Davies: Okay. So, in an antibody, you've got this here, and say this is the light chain and this is the heavy chain. Okay? This is the FAB. This is the antigen binding. And you've got a hinge, so this is VL, this is VH, and this is the binding site. This is CL and this is CH1. And then, coming out from here, you have a hinge. Okay? This is like a hinge coming down this way, and the hinge is characterized by having disulfide bridges and things in it, a lot of proline in it. And then up here you have this other FAB binding site, and you have VL and CL and CH1 and VH. And then down here you have another domain, which is called CH2, and then these adjoined to the third domain, CH3, like this. So this part is called the FC, this part is called the hinge, and Porter, Rodney Porter of Oxford, had shown that if you cleaved--you could cleave antibodies--and if you did, you got two FABs and one FC. And what you were doing was you were cleaving this relatively unstructured region here. Now, the Dobb protein that we worked on looked over looked a bit like this. Let's see. So, here was the FC here. Here were the FABs. And it turned out--this was work that Lisa Steiner did at MIT--she showed that there was a deletion in the hinge region. This is Dobb. The same thing was true for MCG. It also had--I think it was about a 19-residue deletion. Okay? So this long, flexible tether that you had here was not really here, and there was much less room for this molecule to flop around. And so, that's why we thought it crystallized. And Hooper did this structure, but he couldn't--all he could see was about this much. He couldn't see the rest of the structure, and he couldn't see all these parts down here, see, because, we can assume, because of disorder in the crystal. So, for many, so this one only had these two, this one had all three, and so did MCG. This is going into the '70s now 1973 or so.

Park: Tell me what MCG is.

Davies: MCG was just like Dobb. It was a cryoglobulin from some patient, McGregor something like that. And for a long time, we believed that there were no structures, that it would be very difficult to crystallize an intact antibody with an intact hinge. Now, very recently, in the last six or seven years, that's shown to be wrong, and Lisa Harris and Alex McPherson were able to crystallize several antibodies, and they had all the hinge and everything in them, and they looked more like this one would be pointing this way, one would be pointing this way, and then you have this hinge region, and the FC would be out here somewhere. It was more like what you expect an antibody to look like, but there's a big difference between early 1970s and the late 1990s. So that was the first, our first venture into looking at antibody molecules. And we tried to do more with it, but we never really got it to go to the point where we had a high-resolution structure. I think these days it would be possible to do that, possibly, but we don't have any of the material left, and so we can't go back and see whether cryo-crystallography would help us. But at that time, we did the best we could. About that time, Brian Matthews left.

Park: When was that?

Davies: About '69. He went to Oregon. And I had a postdoc called David Siegel, who was a wonderful guy. He got his degree at Hopkins with Bill Harrington, whom I knew very well, and then he went to the Weitzman Institute for a postdoc, and then he came to work with me. He wasn't a crystallographer. He was more of a protein chemist. And he was wonderful....just terrific. And he worked on--we had a structure for gamma chymotrypsin by then, and he worked on the structure and we did various inhibitor-binding studies and things like this. It was all very, very interesting. Then we decided, and I think it was David's suggestion, that we would contact Mike Potter, who worked in the Cancer Institute, and try and get him to give us some material, FABs preferably, of antibodies that had known binding specificity. In order to explain this, I have to go back a step. At that time, there were no monoclonal antibodies in the sense that you could just make your own monoclonal antibodies. Cesar Milstein was the name of the guy who worked with him. And so the only source of real sort of mono, I mean, homogeneous antibody was from patients who had multiple myeloma. You could take the blood and plasmapheresis it, you spun it down and took out the protein and then re-suspended the cells and gave them back to the patient--at least I think that's what they did. And so, Mike had these myeloma proteins that he was able to get in mice. He also gave a talk at this 70th birthday thing. He found that if you injected mineral oil into the peritoneal cavity of the mouse, then the mice would--BALB/c mice-- would develop tumors, and many of these tumors were plasmacytomas. They made cells that were secreting antibodies in large amounts, and it was a single-antibody species that was being made. And, furthermore, you'd have to check with Mike about this--he's still around; I saw him this morning, as a matter of fact, bent low over his bicycle, cycling in. He lives off of Cedar Lane and I occasionally see him in the mornings. And he and various other people were able to identify antigens, not so much the antigen as the hapten [sp.]. Do you know the difference between the antigen and hapten?

Park: No.

Davies: No. The antigen is the whole molecule of a large molecule that tryptin binds to the antibody.

Park: Yes.

Davies: The hapten is the actual determining part of the antigen that binds.

Park: Kind of functional group?

Davies: It's the thing that most interacts with the antibody. And in this case, he had antibodies to phosphocholine, which were believed to be antibodies to a much more complex compound than to just phosphocholine, but they did bind phosphocholine. And so we decided that we would try to crystallize those, that one, to see what it did, and we did. David Siegel was able to do that, and we started working with crystallography.

Park: It's very interesting. If I interrupt your story, it's very interesting that the first time when you were interested in antibody, it was Bill Terry who took your course and Bill Terry was from NCI. Is he an M.D.?

Davies: Oh, yes. . He's, I think, a sort of director or assistant director or something of the Brigham and Women's Hospital in Boston.

Park: And he asked you to examine these, that kind of exchange of information, encouraging research areas and things like that, and you also mentioned that Potter.

Davies: Mike Potter.

Park: Mike Potter and others. You asked to give me the crystalline form of antibody. And it sounds like there is no real area between institutions like NCI.

Davies: No. I've collaborated with many people through the NIH. And, again, the symposium that we had two years ago, there were--most of those people were people I'd collaborated with. So, it was interesting.

Park: Yes.

Davies: John Kendrew wasn't here, but that's because that same day, they were having a symposium for him in Heidelberg. He was the director of the EMBO lab. But most of those people--Ira Pastan, Mike Potter, all sorts of people--I'd actually worked with quite a bit.

Park: Is that kind of interaction the same these days, do you think?

Davies: Oh, yes, very nice. I think so. We have had a long collaboration with Edith Miles, who is in Building 8, on the structure of bi-functional protein, two enzymes glued together, and that's gone on for about since 1986, and she's the wife of Todd Miles, who works in our lab, so she has sort of related to us more closely than otherwise. But we tried for many years to get crystals of her protein. Eventually, she and her co-worker were able to get crystals. And that's turned out to be a very interesting subject to study in detail. She was a wonderful collaborator. And now, for instance, we have collaboration--we collaborate with anybody. In France, they would have strung me up at the end of the war! We have a collaboration now with Reed Wickner who has a yeast prion protein. You know what that is?

Park: No.

Davies: You don't? Well, you're going to have to brush up on your biology. The prions are the things that are involved in these amyloid diseases like Alzheimer's or mad cow disease or things like this, and there's a lot of interest in trying to find out what makes them go this way. It's beginning to be understood. But in Reed's case, this is in yeast, so they have a functional assay right away for when this thing becomes a prion protein, and I think it offers a very attractive system to work with. And one of his postdocs is working closely with some of the people over here to try and get crystals of that, look at the crystal structure. What else do we have?

One of the longest collaborations I have is with Bob Craigie who is in our lab, and he and I have worked on these structures of the HIV integrase. And without Bob, I couldn't have done this work, whereas I'm sure Bob could have found someone else to collaborate with. I mean, he has collaborated occasionally with the NMR people, and the sort of things we do in NMR is not very satisfactory. I'm also collaborating with someone at the Dental Institute, Edie Wolf, and Myung Hee Park.

Park: Sounds like a Korean name.

Davies: I thought you might recognize it. You don't know her.

Park: No.

Davies: And I collaborate with Helen Petrovsky [sp.]. I'm not sure which institute Helen's in. It could be the Cancer Institute. I'm collaborating with Edith Miles, with Bob Craigie, and we've done a lot recently, a lot of things that were of interest that came out of the lab itself.

Park: How was that kind of collaboration initiated? Just personal contact or exchange?

Davies: They'd come to me.

Park: Informal meetings?

Davies: They'd come to me and want to collaborate. They say, "I've got this, the most exciting protein in the world." And it's good for the postdocs, too. I mean, I have a lot of collaborations right now which have really resulted from postdocs beginning to feel uneasy about working on just one problem, particularly if that problem doesn't seem to be going anywhere very fast. And they like to have at least some backup so that they can at least have a paper or two along the way. Of course, what usually happens is that they move over to the new field and forget. That's the down side of it. But people say that the NIH is a great place for long-term research.

But you've got to be able to keep people... I mean, you're dealing with postdocs, visiting fellows, and so on, and fellows, and they have to get jobs. And so you have a double responsibility, and you can't give them things that don't go anywhere in three or four years. They get very unhappy.

Park: Right. So you can't give kind of risky...

Davies: You try to mix risky things with the more surefire things.

Park: That's very interesting. Going back to the 1960s and '70s, certainly you had postdocs, postdoc fellows and technicians. Could you give me the size of your laboratory?

Davies: Oh, very small, very small, yes. I don't think we ever had more than about four or five people.

Park: Including technicians?

Davies: We had one technician. When we moved over there, we hired one technician, Hazel Braxton, and I had... Of course, at that time Marty Gellert was in my section, but Marty worked on his own projects. He was totally independent from me. And Cathy Skinner was a mineralogist. See, there was nobody trained in the field of protein crystallography, nobody in the early days. So this is 1961. And then I had a man called Paul Sigler who was a wonderful guy, absolutely wonderful. He was an M.D., and it was during the time of the doctors' draft.

Park: The Korean War?

Davies: The Korean War. We have to thank the Korean War for these guys. I mean, that had a huge impact on the quality of people who came to the NIH. And so, Paul--he had a big, deep voice, very loud, a big man, and he worked first of all on a polynucleotide problem I gave him, and then he worked on gamma-chymotrypsin. And he came over with us from Building 10 and stayed for about two years over in Building 2. Then he went to Cambridge. He'd already interned and done his residency, so he was a qualified M.D. to go out and do things. And when he first came through for an interview, he talked only about his medical experiences. And at the end of about half an hour, I said, "Well, you know, Paul, if you come to work with me, you'll probably never see another patient," because this is so divorced from clinical medicine. But he wouldn't listen. He wanted to come, and so he came, and he was wonderful.

Park: How much did he help in science?

Davies: Oh, his chemistry was terrific, and he had a very low barrier to jumping into any field. I mean, he could do anything. He was smart and capable.

I won't say anything negative about him. He was really a great guy. I still like him very much. He's at Yale now. He went to Cambridge, got a degree with David Blow. He worked on the other chymotrypsin, the alpha chymotrypsin. And it was he who suggested to Brian Matthews that Brian come to my lab as a postdoc, and that was terrific too. But Paul went to, after Cambridge, he went to Chicago, and he was there for many years, and he was doing pretty well. But I didn't think that he ever did as well as the potential that he'd shown, justified the potential that he'd shown when he was with me. And then he moved to Yale and became a Hughes investigator about seven or eight years ago, and he's taken off like a rocket. He is the world's most visible, or almost, you know. He's among the group, the top 10 in the world, of the most-achieving crystallographers at the moment, and I'm very impressed. And I think a lot of it must have to do with the different students and access. I was very pleased for him.

Park: So, in terms of the size.

Davies: So we never had large groups. I would have liked to have tried to keep Brian Matthews on in the lab because he was so good, but I couldn't get Stetten to agree to that. It was very difficult. Resources were much more limited than they are now. And then, by the mid-'60s, I hired Gerson Cohen, who's still here, and Enid Silverton. Now, Gerson came as a fellow, and I had plenty of time. We were just starting the fellowship business at that time. That's what really saved the NIH from becoming ossified, because up to that point, anyone who worked here for a year would have civil service tenure, and that would have killed the NIH. I don't know who initiated it all. It was probably under Shannon. But the whole system of having fellows and only bringing people in on relatively short-term contracts was what saved us. So Gerson came in and we liked him, and he's now--of course, he's been here ever since. Enid came in, and she had tenure already. She had worked down at the Bureau of Standards for two years, and so she had tenure. And they joined the lab, and Brian was here in the late '60s. Sarma came in the late '60s and worked on--he'd worked with David Phillips on lysine in London, and he worked on the G protein. He was able to get some sort of electron density map that we could roughly interpret. So those were the small groups. I mean, there were maybe four or five, at most, of us.

Park: Still, you have a small group in your section.

Davies: Yes. I have a particularly small group right now because right now. Well, it's bigger than you think--okay--the section. Just take the section. The section has me, I have two postdocs, I have Gerson Cohen. Then there's Fred Dyda. Fred's in my immediate section. He's a staff scientist. Fred works with Allison Hickman, who's--I think she's a staff scientist, and they have a postdoc, so there's a comparable group there. And then there's Eduardo Padlan down the hall. Eduardo does his own work. Fred and Allison do their own work, and Eduardo does his own work, and he's working on antibodies and he has one postdoc at the moment. And then, also, in my section, there's Bei Yang. Have you heard of them before?

Park: No. I just saw the Web page.

Davies: Right. She's down in the basement, and she's doing absolutely fantastic work. And then we have, upstairs we have Jim Hurley, who works in signal transduction, and he's been very successful and he's got tenure, and we've given him his own section now, and he's expanding. I mean, he's an expansionist, quite different from the way the rest of us were.

Park: I see. I see what you mean by saying that your section is bigger than I thought. Yeah.

Davies: Right, because administratively I'm responsible. But, in fact, Rae does her own research, she is entirely responsible for her own program. She has access to the x-ray equipment, but she chooses her own research subjects and does whatever she decides is necessary to work out the structures, similarly with Eduardo and with Fred.

Park: Back in the 1960s and '70s, did you ever try to expand your laboratory like Pauling's laboratory?

Davies: No. We were too involved in trying to get these structures out because the rise of protein crystallography pretty well parallels the rise of computers and also of data collection devices. And, you know, we used to do everything. In Perutz's day, when we started, it was enormously labor intensive. You had to--if you wanted to collect the data set from the protein crystal, you only had these things that were called procession cameras. It was a camera that took a plane out of the reciprocal space of the crystal and gave you all the intensities that lay on that plane. And so you had photographs which were just covered with spots, and you had to use a manually driven microdensitometer that would record these intensities. So, very often you'd overexpose, you'd have to use several films to get a range of exposures, and it was tedious. I mean, it was very tedious just measuring these things. And then what you got was a tracing of what the intensities looked like, and then you had to measure each one with a ruler. I'm not kidding. And then those had to be put onto magnetic tape or onto punched cards, and then the punched cards had to be taken over to the computer, all the corrections had to be applied to them. It was a huge job. And Gerson Cohen can remember much more about those things than I can tell you, because he's always been involved with our computers. So, nowadays, you take a crystal, put it on the machine upstairs, use cryo-crystallography so that the crystal doesn't die. The other thing was that when we were doing this at room temperature, the crystals died in 24 hours, or at least they began to die, so you had to make corrections for that or put in new crystals. And if you had new crystals, you had different absorption coefficients, and so you had different ways of correlating the data. It was a mess. And so nowadays, you take one crystal. You used to have to do crystals in glass capillaries, and there would be a little bit of liquid in there as well to keep them moist. Now what you do is you put them in a little nylon loop, and they're suspended in here with just a tiny amount of liquid, so the absorption is very small.

And now you have automatic machines. First we had diffractometers where you had a single detector that moved when the crystal moved. You collected the data from one spot and then you moved, collected data from another spot. Well, by the time you'd worked for 24 hours, your crystal was in bad shape. So then you'd put another crystal on and you'd start again and do all this. It took a long time to collect data. And then once you had it, you didn't really have the computers at the beginning that you could use to interpret all this quickly. And it's just totally different now. Everyone who comes here now as a postdoc is very sophisticated in the use of computers.

Park: Once you mentioned that you used the computer available in the registrar's office.

Davies: This was in what is, what became DCRT, yes.

Park: And after that, how did they help you to use computers?

Davies: Well, for a while, we would do all our calculations over at the central computer, and I'm a bit fuzzy about the dates on this, but, again, Gerson Cohen can tell you more precisely. But gradually, they got more and more expensive because we were using a lot of computers and computing time.

Park: Were the x-ray crystallographers most demanding?

Davies: Could be, yes, at that time certainly. So we decided to get our own and we've never looked back. We've always had our own computer since then. I think it was a sort of universal experience. The big core computer went out of fashion, and, except for the sort of massive things like the Cray and so on, because they were too expensive and it was cheaper to buy something that was dedicated to what you wanted to do. But even then, it took a long time. Most of the people who come here now as postdocs are totally sophisticated in computers. I mean, they know everything there is to know. I find myself a bit of an ignoramus.

Park: One day, one of your postdocs...

Davies: Tim?

Park: Yeah, Tim showed me how to observe crystals from computer screen, and changing the angle and upside-down and right and left, and you can see every angle.

Davies: Sure. Was it a crystal or a protein molecule?

Park: I think a protein molecule.

Davies: Yeah, yeah.

Park: It was fantastic! We could never see such things.

Davies: Well, see, one thing that we used to do--you've seen those models over in the museum; we've got another one downstairs too. Are you interested in another one of those models?

Park: Oh, yes.

Davies: For the museum?

Park: Yes.

Davies: We've got one for the gamma chymotrypsin downstairs. It's just sitting there. I could dress it up to make it look very nice, so ask Victoria if she wants to...

Park: Okay, yes.

Davies: Wants to have that. She can have it because they need to put in a freezer down there.

Park: Great, great.

Davies: If not, we probably will tear it down, but I hate to tear it down if there' it took such a long time. Now, those things would take months to build.

Then, gradually, people began to devise programs that would help you to build it on the computer. And nowadays, it's just trivial. I mean, you have--there are all sorts of programs that enable you to follow an electron density map and put in the polypeptide chain. It's just quite trivial. Well, I shouldn't say that. I think of it as being trivial compared with what it used to be. So that, in those days, everything you did was sort of new.

There was no local knowledge. I mean, maybe somebody in Cambridge had done it before, but, and we knew roughly what they'd done, but you'd really have to work out your own strategy for looking at protein structures. And when you undertook a particular protein, you never really knew that you were going to be successful in solving the structure. Nowadays, that's not the case at all. I mean, in those days, you knew what everybody else in the field was doing, so you knew, you know, if Dickerson was working on lysozyme and somebody else was working on cytochrome and Joe Kraut was working on, well, he eventually picked up subtilisin. He was working on subtilisin. And David Rowe [sp.] is working on alpha chymotrypsin, and you knew all that and you didn't work on a problem.

You tried not to work... I mean, the alpha and the gamma was an unfortunate thing that they turned out to be so similar. But you tried not to work on the same problem as somebody else because it was counterproductive.

Park: Right.

Davies: And, nowadays, you have absolutely no idea. In those days, it took maybe six or seven, five, six, seven, 10 years sometimes to work out a structure. Nowadays, it takes three months.

Park: Three months.

Davies: Once you've got a crystal. And so, you have no idea, you know. There are high-visibility projects. Lots of people work on them. You're apt to be scooped anytime. It's a much more competitive field these days than it was at that time. It was a gentleman's field, what we used to call it, because everybody knew everybody else and we were all very polite to one another.

Park: Do you know when was the transition period?

Davies: Well, the transition is still going on. I mean, it's changing as we speak, all the time, but certainly the situation changed enormously then. Nowadays, most people, I think if they thought there was an interesting problem that they could contribute to, would have no hesitation working on a protein that somebody else had worked on.

Park: Do you know the Korean, not sure of the name?

Davies: He went to Cornell the same time as Gerson Cohen.

Park: And he was one of the first who made a program for protein, a computer program for protein structure. Did you use that kind of program?

Davies: The sort of early problems that we had, well, one of them--again, Gerson is the person to check with on this--was a program by Bob Diamond from Cambridge. And then, later, Alvin Jones was another Welshman who worked in Munich for a while with Robert Hoover [sp.]. I think he's a mathematician originally, though he's become a very well-known figure in the field of crystallography and now works at Uppsala. He developed a program, software that most people use these days. There are lots of programs around now. I don't remember B.K.'s program.

Park: I see. I was just interested in how the computer division here at NIH was helping others in terms of using computers.

Davies: No, no. You'd get your own software, you brought in your own programs. We never did anything... I told you my experience with the helical diffraction patterns, I mean, that I had to do the... I had to wire the plugboards myself in order to get it to work. It was a lot of fun, but there was no one there who really knew enough to be able to do that. And most of the algorithms that have the software packages that have been derived have come from people who are professionals in the field of protein crystallography.

Park: Going back to your antibody studies in the early 1970s, could you pick one of two papers which are really important in terms of your antibody studies?

Davies: Already, in 1968, we were getting along with gamma chym [sp.], but we weren't nearly as close as the alpha chymotrypsin people. Here it is. That was the first protein structure that we worked on. It took many years before we finally published the actual structure, but that was because of-- Gerson [sp.] did the refinement, and refinement procedures were just coming into being, and he decided to try and refine it as much as possible. Wayne Hendrickson, who is now at Columbia, worked for Jerry Karle in Jerry Karle's lab. You know Jerome Karle?

Park: No.

Davies: Jerry Karle got the Nobel Prize in crystallography for developing direct methods for solving small molecule structures. And he works at Naval Research Labs here in Washington. And Wayne Hendrickson, who is a very well-known crystallographer, worked in the same, worked in Jerry's lab. And whilst he was there, he collaborated with a man called John Konnert and they developed this software for refining the structures of proteins by putting in lots of constraints so that you couldn't bend. You know, if a bond was known to be planar, then you couldn't bend it very much out of shape and that sort of thing. And allowing the psi angles... Are you familiar with those?

Park: Not really.

Davies: Well, if you have two peptide groups... Let's just draw something like this--so this is a carbonyl and this is a C alpha and this is an NH. Okay? So this group forms a plane. They're constrained to lie in a plane. Pauling showed that. And this group is confined to a plane. So what you have is you can rotate about this bond or you can rotate about this bond. Okay?

Park: Okay.

Davies: And rotate about both of them. And one is called phi and the other is called psi. Okay? And you can, by varying these or, say, by measuring the phi/psi values at each alpha carbon--so you'd have another one here, there'd be another phi here and another one over here, and so on--by measuring all the phi/psi's, you define the shape of the polypeptide chain. So, that's what these programs did. I mean, they kept things, you know, reasonably rigid, and if you wanted to vary things, you could vary them some, but you couldn't vary them all that much. And so it took a long time to apply this. People were just beginning to think that protein structures could be refined, but, in general, there weren't enough reflections, that is, independent observational measurements, to fix the positions of all these thousands of atoms. And, say you have, you know, chymotrypsin or something. It had something like 250 amino acid residues, and each residue had maybe, say, on the average, seven atoms associated with it, so that's about 1,500 or so atoms without the hydrogens, and the amount of data that you collected wasn't enough to enable you to refine solely based on the data because there would be too many independent coordinates that you were trying to determine. But by linking them in this way, you put constraints onto them, and you paid a penalty if you made it too rigid, but, at the same time, introduced a number of things that you were trying to measure to a point where you could actually do this. And so this was, again, Gerson is the person to talk to. He's just across the hall. So, let's look at this. See how involved in nucleic acids since 1971. This could have been great. We've got crystals of tRNA and that could have been a great crystallization. A lot of it is technology. This is the first one. Okay? I like this. There was a guy in Building 2 who worked for the other lab. His name was Louis Labaw, and I think I talked about Ralph Wyckoff.

Park: A little bit, not much.

Davies: Yes, Ralph Wyckoff. He was the senior crystallographer who was in Building 2, whom I never really met, and I think he was gone by the time we moved in there.

Park: He worked with small molecules.

Davies: He worked on small molecules. And one of the people who'd worked with him was this man, Louis Labaw. His name was L-a-b-a-w. And he was an electro-microscopist. And what he did was, he had these crystals and we had a tentative structure. We had an electron density map and we could plausibly account for it in terms of the structure of the antibody, but we didn't know any of the details, so... And it's something like this. So, what he did was, he affixed the crystals by putting in pure aldehyde and just cross-mix the different proteins, so it's like rubber. And then he cut a section, and he cut the section parallel to the plane of the paper, perpendicular to the plane of the molecule. And then, so he gets a picture. Then he put it in the electron microscope. He gets a picture that looks like a very poorly plowed field, literally. I mean, you can see the traces of lines going around this way and this way, but you really can't see very much. And it wasn't really high-class electron microscopy, but it was interesting. And we assumed that the repeats that you were seeing were crystal structure repeats. So then what he did was he took the negative of those and he put it in an enlarger and projected it down onto the floor, and then he took a piece, a large piece of printing paper and he made an exposure of this image down onto it. Then he moved the print by one step. You know, he had all these lines, and so he moved it from here to here and took another exposure. And then he did this seven or eight times, or maybe even more, and what happened was that all of a sudden he could see these little guys sitting there like this. You know, it's like the soldiers in Xian. You could see them all lined up. It's really something. You've not seen that. I'll have to show you that paper. He's dead now, unfortunately. But that was a very interesting paper. Then we got crystals of FABs. Now, this was an important paper, but there was also a pattern on paper. So we got a low-resolution structure, and then this was a high-resolution structure. We had been scooped because Robert Polyak worked on another antibody which didn't have binding properties, and he worked that one out. He published it in 1973, right at the end of '73, and Alan Edmondson [sp.] published a structure of a Bergstrom's protein. Do you know what a Bergstrom's protein is?

Park: No.

Davies: Bergstrom, I think he used to be an English physician, and for people who have multiple myeloma, they secrete very often with lots of light chains, and Bergstrom proteins are light chain, usually dimers. And his paper was actually the first. It was Edmondson and Marianne Schiffer. And they had this MCG light chain, and this showed clearly what the domain structure was. This was the first demonstration of that. It was for a Bergstrom's protein. And we came out a year later.

Park: Where were they?

Davies: They were in Argonne.

Park: Argonne.

Davies: Yeah. Let's see.

Park: So that field was very competitive.

Davies: It was quite competitive. I mean, these papers look trivial now. They're the sort of thing you do in a few days. We sweated over those. This was a very good paper, and this paper I thought was very interesting. We're getting into '75, yes? This was quite an interesting paper, too. The things keep on creeping in. In 1975, I was going back to nucleic acids. This was an early paper. This paper was an interesting paper, it must be about '77. By that time, by 1975 or so, people pretty well knew all there was to know, at that time at least, about the serine proteases. Do you know what these things are?

Park: No.

Davies: That's the trouble with this. There are so many technical details. So, serine proteases are characterized by having something that's CH₂OH, serine, by having a histidine--we should put that down here--histidine ring system that's there to accept a proton off the serine, and so let's do it like this. And then this is held in position by a carboxyl group, which is an HIS. So you have HIS-ASP-serine, and that's something that was originally called the catalytic triad, and that's the thing, plus the two other little things, that's the same in gamma trypsin and trypsin and all these things, and you actually find the same arrangement of these residues in subtilisin even though the subtilisin structure is quite different. Trypsin and gamma trypsin are very similar. So, but there are other classes of proteases. There are four classes altogether, there are lots of these. Renin, which controls blood pressure. So, also, many fungal classes. So, we worked on the fungal protease. The only thing we knew about it was that it was called an acid protease because it worked at low pH, and it was believed to be created by the fungus to dissolve the around it so it couldn't have a good meal. We never published it. Anyway, I had this very nice English postdoc, Ian Swan who crystallized this and then he left. And I went on sabbatical. It was the worst year for sabbaticals in my life. I should never have done it. I went to Heidelberg, and the antibody work was still going on. Eduardo and David Segal were working on that, and I was essentially out of touch for a year. It was terrible. Ian Swan had calculated enough for this thing, but we couldn't interpret it. I took it to Germany with me, but I couldn't interpret it. And then he went back to England and worked on it for the next few years, and eventually we got a structure for it, and that's this paper here.

Park: Number 63.

Davies: Right. This is 63, right. And it turned out that we didn't have a sequence. Now, if you have very good data and very sophisticated ways of defining a structure, you might be able to guess most of the sequence from the crystallography these days, but it certainly helps to have a sequence. And he--we just didn't have the sequence. And so, eventually--boy, this thing sat around for a long time. I went to a meeting in Oklahoma, which was organized by Jordan Tang who is from China originally, and he had sequenced pepsin and he did it by amino-acid sequencing. It took a long time. It was a big achievement to do that. Jordan is very smart. And so he organized this meeting. And at the same time, Tom Blundell, who is now in Cambridge, and Mike James, who's at Edmonton, and we all had structures for different fungal proteases. Mike James had a structure for penicillin pepsin. And, in fact, I arranged that. We went on a site visit.

There was a man called Theo Hoffman [sp.] who was working in Chicago, and Theo, we went on a site visit, and Theo was collaborating with some crystallographer, small-molecule crystallographer. Did I say in Chicago? In Toronto. And so, it was Al Stravinsky [sp.] and me, I remember. And we recommended that the project be taken away from his collaborator and that he collaborated with Mike James. And Mike has made his living off these things. I mean, other things too. He's a very good crystallographer, but it's made a big impact on his career, and so we're very pleased with that. And so he had penicillo pepsin and we had pepsin, and Tom Blundell pepsin. But we didn't have a sequence. He had a sequence, and so he had good data, and so he got ahead of us at that point, although we published more or less at the same time. But he was able to see more details. Well, these proteases, these spotle proteases, are very interesting. They have two carboxyl groups in the active site. There's the substrate, which is a peptide, binds in the active site, and then there's a various exchange of protons and so on, and that's how catalysis occurs. They all bind. They're all inhibited. They bind to and are inhibited by a protein or a peptide called pepstatin from streptomyces. And at the same time, at this meeting, there were [names unintelligible] working on pepsin, and presented a structure for pepsin and we presented structures for each of these, and ours were all the same and the others were different. So we had to note that we thought there was a misinterpretation, and they got the right structure. So this is another ubiquitous class of enzymes, super-family of enzyme. I mean, you find them all over the place. You find them in lysosomes. You now find them in the proteins that are anchored in the membrane that make the cleavage for amyloid diseases like Alzheimer's. So they're a very important class of proteases. And what we were able to do was to get the sequence. There was a Japanese guy who was supposed to be doing the sequence, and it took forever. I mean, he wouldn't let Jordan do it, Jordan Tang, and it just took forever. It took 10 years. And then, when we got the sequence, we could then do the structure properly, and we were able to do the activity and propose the mechanism for action and everything else. But that didn't happen until 1987.

Park: Number 91.

Davies: Yes, and this one.

Park: Ninety-two.

Davies: Now, one of the reasons that I'm so interested in all this, and one of the reasons I got into the AIDS business, is that this structure was, is, I mean, the HIV protease is an aspartic protease, and I wanted to work on that when the AIDS thing first came along because they had a meeting to discuss whether we should have an intramural program on AIDS. It must have been about 1987 or so. And it was organized by some of the Building 1 administrators, and it was a very good suggestion, and have a group that would focus perhaps a little bit more on the structure and biology rather than just the biochemistry. And I used as an example for supporting this the fact that this protease was almost certainly an aspartic protease. Not very much was known about it at that time. But it would have to be a *dimer* because it was too small to be a *monomer*, and it had the characteristic signature sequence of the aspartic protease and it only had it once whereas all these others had it twice because they were a single polypeptide chain. So all this was understood, and I said, you know, this will bind; this will almost certainly bind to pepstatin because pepstatin binds to everything. So, after the talk, Sam Broder came up, and he was the, I guess he was the director of the Cancer. No. He was probably the clinical director of the Cancer Institute. And he said, "Could you get me some pepstatin?" so I said, "Sure." So I gave him some pepstatin and he tried it out.

End of Transcript